IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Patent Application of: Applicants: Sierra *et al.* Serial No.: 10/003,462 Filed: December 6, 2001 Title: VACCINE COMPOSITION CONTAINING TRANSFORMING GROWTH FACTOR ALPHA (TGFα). ITS USE IN MALIGNANT DISEASES THERAPY Docket No.: 30797-717.201	Group Art Unit: 1643 Confirmation No.: 4354 Examiner: Anne Holleran **Certificate of Electronic Filing** I hereby certify that the attached **Response to the Office Action dated November 20, 2006** and all marked attachments are being deposited by Electronic Filing on **May 18, 2007** by using the EFS – Web patent filing system and addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450. By: Norman Green **Norman Green**

Declaration Under 37 CFR § 1.131 of Belinda Sánchez Ramírez

Mail Stop Amendment
Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, Belinda Sánchez Ramírez, of Havana, Cuba, hereby declare as follows:

1. I am the Head of Recombinant Vaccines Group and a researcher at the Centro de Inmunologia Molecular and an inventor of the present application. I have been conducting research in fusion proteins and tumor immunology for over 11 years. Accordingly, my *Curriculum Vitae* is attached herewith as **Exhibit A**.

2. I have read the specification of the above-identified application, the pending claims and the Office Action mailed by the USPTO on November 20, 2006.

3. I understand that the Examiner has rejected the claims as allegedly being obvious in view of several references, two of which are Gonzalez (Gonzalez et al. Scandinavian J. Immunol., 52: 113, **August 2000**) and De Luca (De Luca et al., Oncogene, 19(51): 5863-5871, **November 2000**).

4. These references were published less than a year before the filing date of priority application CUBA 286/2000, filed December 6, 2000.

5. The compositions as disclosed and claimed in the present application were conceived and reduced to practice prior to August 2000.

6. As evidence of this, attached herewith as **Exhibit B** is a laboratory notebook page exemplifying the protocol used to generate P64-TGFα fusion protein as described in Examples 2 and 3 of the present application.

Briefly, the expression vector pM 92 was used. The plasmid contains the IpdA gene coding for P64k protein from *Neisseria meningitidis* (strain B385) under the control of *E. coli* tryptophan operon promoter (ptrp) and phage T4 transcriptional terminator (tT4). pM 92 contains ampicillin (Amp^R) and kanamycin (Km^R) antibiotic resistance expression cassettes. The pM92 vector was digested and subsequently ligated with the cDNA from TGFα.

The resulting plasmid codes for the fusion protein that contains hTGFα inserted among the amino acid 45/46 of P64k and containing a polyHis sequence.

FIG. 2 of the present application shows a schematic representation of the expression vector obtaining process. This vector codes for the fusion protein TGFα-P64K which was made using techniques described in the laboratory notebook page and herein.

7. In summary, the laboratory notebook page presented herein illustrates that the TGFα-P64K fusion protein compositions as presently claimed were conceived and reduced to practice prior to August 2000.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false settlements and the like so made are punishable by fine or imprisonment, or both, under Section § 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Belinda Sánchez Ramírez

Date: 17/Mayo/2007 Signature:

EXHIBIT A

Application Serial No.: 10/003,462

Attorney Docket No.: 30797-717.201

Curriculum Vitae

Personal data

Name: Belinda Sánchez Ramírez
Date of birth: January 30, 1970
Citizenship: Cuban
Home address: 184 Street between 1st and 5th Ave, Flores, Playa
Havana 11600, Cuba

Present position: Researcher of Vaccine Department.
Head of Recombinant Vaccines Group
Research and Development Direction.

Present Address: Center of Molecular Immunology (CIM), 216 Street and 15,
Atabey, Playa. Havana 11600, P.O. Box 16040, Cuba.
Telephone: 537 217645 Fax: 537 335049
E.mail: belinda@ict.cim.sld.cu

Education: BS in Biochemistry with Distinction (Faculty of Biology, Havana University, 1987-1992).

Profesional Experience: Center of Molecular Immunology (1992 – present).

- Technical experience in biochemical techniques (SDS-PAGE, Western Blott), Molecular Biology, cell culture, protein purification.
- Generation of a cancer vaccine based on human EGF, chemically conjugated to P64k from *Neisseria Meningitidis* and evaluation in preclinical studies (CIM).
- Cloning, expression and purification of a fusion protein human EGF-P64k from *Neisseria Meningitidis* for the formulation of cancer vaccine. Evaluation of the immunogenicity in preclinical studies (CIM).
- Cloning, expression in mammalian cells and purification of extracellular domain of murine EGFR (mEGFR-ECD) and human EGF receptor (HER1-ECD). (Max-Plank Institute for Biochemistry, Germany).
- Generation of a cancer vaccine based on mEGFR-ECD and Her1-ECD adjuvated on very small size proteoliposomes from *Neisseria Meningitidis* . Evaluation of the humoral and cellular imunogenicity and antitumoral effect in preclinical studies (CIM)

Post-Graduate Studies

- Molecular Immunology course (CIM / University of Havana, 1992).
- Applied Biotechnology Course (CIM / University of Havana, 1993).
- Immunology Course (CIGB, 1993).
- Quality Control and Statistic (CIM / ISPJAE, 1994)
- Good manufacturing Practices (CIM, 1994).
- Immunology Course (CIM, 1995).
- English Course (University of Havana, 1996).
- German Course (Goethe Institute, Freiburg, Germany, 1996).
- Molecular Oncology Course (Havana, Cuba, 2000)
- Introduction to proteomic (Havana, Cuba, 2006)

Participation in meetings and Congress:

- Biotechnology Congress Havana'92. CIGB, Havana, Cuba, 1992.
- First International Symposium about Encephalic Death. Nefrology Institute, Havana, Cuba, 1992.
- Second national Workshop of Cellular Immunity. Finlay Institute, Havana, Cuba, 1994. Author.
- International Workshop "Immunotherapy in the Nineties". CIM, Havana, Cuba, 1994.
- XII Scientific Seminar "Cancer Immunology and Immunotherapy ". CNIC, Havana, Cuba, 1995. Author.
- International Workshop "Immunotherapy in the Nineties". CIM, Havana, Cuba, 1996. Co-author.
- International Workshop Biotehcnology 1997 "Medical applications of biotechnology" (CIGB, Cuba, 1997)
- XI Forum of Science and Technology, Havana, Cuba, 1997
- Forum of Science and Technique. CIM, Havana, Cuba, 1997 and 1998. Co-author.
- "Cancer Vaccines'98" Conference. Bethesda MD, USA. Co-author.
- XIII Latin-American Integrated Congress of Cancerology. Havana, Cuba, 1999. Author and co-author.
- Workshop "Immunotherapy for the New Century, Havana, Cuba, 2000.
- 6to Latin-American Congress of Immunology. Havana, Cuba, 2002
- International Workshop: Immunotherapy for the New Century, Cuba, 2002.
- Biotechnology Congress Havana´2003. Havana, Cuba, 2003.
- International Workshop: Immunotherapy for the New Century, Cuba, 2004.
- Cancer Vaccines/Adjuvants/Delivery for the Next Decade Congress(CVADD), Portugal, 2005.
- Cuban National Immunology Congress, Cuba, 2006.
- International Workshop: Immunotherapy for the New Century, Cuba, 2006
- Workshop UCL-CIM, 2007

Docent experience

- Professor of Advanced Molecular Immunology Course in 2002, 2004, 2006 and 2007.
- Supervisor of diploma thesis in 2001 and 2006.

Publications and patents

- González,G., Sánchez,B., Suárez,E., Beausolei I., Pérez,O., Lastre,M., Lage, A. (1996) Induction of Immune Recognition of Self Epidermal Growth Factor (EGF): Effect on EGF Biodistribution and Tumor Growth. Vaccine Research 5(4): 233-244

- González,G., *Pardo,O.,* Sánchez,B., Beausolei I., Lage, A. (1997) Induction of Immune Recognition of Self Epidermal Growth Factor II: Characterization of the Antibody Response and the Use of a Fusion Protein. Vacine Research 6 (2): 91-100

- González,G., Sánchez,B., Beausolei I., Suárez,E., Lage, A. (1997) EGF Based Cancer Vaccine. Biotecnología Aplicada Vol 14, No 1

- Suárez, E., Greiser, U., Sánchez, B., Fernández, L.E., Lage, A., Pérez, R., Böhmer, F.D. (1997) Growth inhibition of Human Lung Adenocarcinoma Cells by Antibodies Against Epidermal

Growth Factor Receptor and by Ganglioside GM3: Involvement of Receptor-Directed Protein tyrosine Phosphatase(s). Br J Cancer. 75(2):213-20

- González,G., Sánchez,B., Beausolei,I., Pardo,O., García,J.L., Crombet,T., Catalá,M., Hernández,J.C., Mirabal,V., González,Y., Marinello,P., Domarco,A., Guillén,G., Pérez,R., Lage.A. (1998) A Novel Vaccine Composed by Human- Recombinant Epidermal Growth Factor linked to a carrier protein: Preclinical Studies and Report of pilot Clinical Trial.. Cancer Vaccine's98 Conference Abstract

- Sánchez,B., González, G., Mulet, A., Guillén, G., Beausolei I., García,J.L., Lage, A. (1999) Effect of the Immunization with Epidermal Growth Factor (EGF) on EGF Biodistribution and Tumor Growth. Use of a Fusion Protein. Use of a fusion protein as immunogen. Oncología, 22 (supl. 1).

- Mulet, A., González, G., Sánchez, B., Crombet, T., García, B., Beausolei I., Lage, A. (1999) Humoral immune response induced by vaccination with human Epidermal Growth Factor. Oncología, 22 (supl. 1).

- Garrido, G., Sanchez, B., Rodríguez, H.M., Lorenzano, P., Alonso, D., Fernández, L.E. (2004). 7A7 MAb: a new tool for the pre-clinical evaluation of EGFR-based therapies. Hybrid Hybridomics.;23(3):168-175

- Sánchez, B., Suárez E., Garrido, G., Hernández T., Pérez, R., Ullrich, A.,
  - Fernández, LE. (2006). Specific Immune Response Induced by Immunization
  - with Self Epidermal Growth Factor Receptor-Extracellular Domain. IJC
  - 119, 2190-2199.
- Garrido, G., Sanchez, B., Pérez R., Fernández, L.E. (2004). Antitumo activity of anti-EGFR 7A7 antibody is not dominated by target expression levels. Applied Biotechnology. In press.

- Garrido, G., Lorenzano, P., Sanchez, B., Beausoleil, I., Alonso, D., Pérez, R., Fernández, L.E. (2007). T cells are crucial for the anti-metastaic effect of anti-epidermal growth factor receptor antibodies.

- Fernández LE, Sánchez B, Mesa C, Suárez E, de la Barrera A. Pharmaceutical compositions enhancing the immunogenicity of poorly immunogenic antigens (2002). No. Pub WO 02/45746

- Lage A, González G, Sánchez B, Suárez E, Beausoleil I, Núñez G. Vaccine composition comprising autologous epidermal growth factor or a fragment or a derivative thereof having anti-tumor activity and use in the therapy of malignant diseases (1997) JP2923519, US 5,894,018, China 95115090,

- Fernández LE, Sánchez B, Suárez E, de la barrera A, Pérez R. Composiciones farmacéuticas para la inmunoterapia específica del cáncer (2004). Cuban patent No 23000.

EXHIBIT B

Application Serial No.: 10/003,462

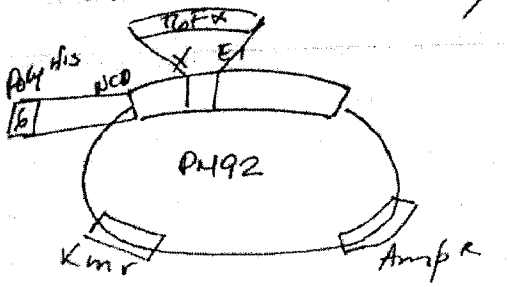
Attorney Docket No.: 30797-717.201

Jueves:

(1) CHEQUEO HIND III Clonaje PM92-T6FX (vector flaco)

[redacted] la cosa quedo ok pues el PM92 debe sacar 2 bandas 3,1 y 2,89 y los clones 2 too 3,1 y 3,00 y en los minis se ve una sola banda (migraron juntas) y en el PM92 se ven 0 bandas.

ojo: El asunto del clonaje del T6F-X se complicó por lo sgte: El objetivo es clonar el T6F-X en XbaI/EcoRI del pM92 y después clonarle una cola de Poly his en el sitio NCOI delante del N-terminal P64K.



y Hasta ahora se tiene clonada la banda del T6FX X/E1 en el PM2 pero [redacted] ∴ no se puede clonar la cola de poly his x digestión simple.

Por lo tanto el otro clonaje será como sigue:

PM92 / NCOI

↓

clonarle cola de poly-his

↓

pM92 (His) / XbaI / EcoRI

↓

Clonarle el T6FX

1- Primer clonaje

pM92 + cola His

Digestión PM92 NCOI

22 ul PM92 6e (v 3 ug)
10 ul b4 NEB
10 ul NCOI (10 u/ul)
58 ul H_2O.
100 uls.